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Pulsed-amperometric detection of urea in blood samples on a conducting polypyrrole–urease biosensor

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Abstract

A rapid and highly sensitive method is described for reliable flow injection analysis of urea based on pulsed-amperometric detection with a polypyrrole–urease biosensor. The optimum conditions were 0.05 M phosphate buffer (pH 7.0), a negative potential pulse between −70 mV (E_1) and −400 mV (E_2) vs Ag/AgCl, a pulse width of 120 mS, and a flow rate of 0.1 ml min^{-1}. A detection limit (3σ) of 60 μg l^{-1} of urea was achieved by this method and the response was linear in the range 100–4500 μg l^{-1}. The biosensor was very stable in the flow system, even after two weeks of continuous use. Excellent recovery of urea (95%–112%) was accomplished employing this method in simulated samples. Successful application of the method to the determination of urea in blood plasma is reported.

Keywords: Amperometry; Urea; Urease; Polypyrrole; Biosensors; Blood plasma; Flow injection

1. Introduction

Previous studies [1–3] have demonstrated that biosensors are useful in improving the selectivity of analytical detection in flow injection systems. The ability of enzymes to catalyse specific reactions is extremely useful in discriminating against sample components which may otherwise interfere. While many reported conducting polymer biosensors [4–8] involve the production or consumption of either hydrogen peroxide or oxygen, which often results in Faradaic responses, several others, e.g. penicillin [9], urea [3,10,11] and human serum albumin [12], employed non-Faradaic reactions, such as adsorption and desorption, or conductivity changes for analyte detection. However, the fabrication of these biosensors by incorporating appropriate enzymes into conducting polymers, such as polypyrrole, enables the amplification of the non-Faradaic response through alteration of the resistance of the conducting polymer during the enzymatic reaction. On the other hand, the accomplished sensitivity and detection limits for these biosensors are often restricted when employed in the potentiometric and dc-amperometric modes of detection.

Recently, we reported [10,11] a simplified approach for direct immobilisation of urease into conducting polypyrrole film and its use for potentiometric detec-

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tion. Unfortunately, the sensitivity of the biosensor was low, requiring further improvement by either increasing the enzyme loading in the polymer or employing amperometric detection [3]. In the latter case, the resulting amperometric signals were due to the alteration of the polymer resistance after the catalytic decomposition of urea.

In this paper, the use of pulsed-amperometric detection is explored for the improvement of the detection limit of a polypyrrole-based urease biosensor for flow injection analysis of urea. The two significant factors considered in improving the sensitivity of the amperometric response and, hence, the detection limit of the method are potential pulse direction and pulse width. Recovery efficiency of the method is investigated with simulated samples. The application of the method to the determination of urea in blood plasma, after the removal of the undesirable anions, is demonstrated.

2. Experimental

2.1. Chemicals and standard solutions

Pyrrole and urease were purchased from the Sigma Chemical company. Pyrrole was distilled under vacuum prior to use and was stored in the refrigerator after covering it with aluminium foil to prevent UV degradation. Urea and other reagents were of analytical-reagent grade and 2.0 M stock solutions were prepared, where necessary, and were diluted later to give the required standard concentration. The urease solution was prepared by dissolving an appropriate amount of urease in water, and then filtered through a 541 Whatman filter paper prior to use.

2.2. Electropolymerisation of polypyrrole–urease film

The electropolymerisation and entrapment of the enzyme was performed galvanostatically by applying a current density of 0.5 mA cm^{-2} to the working electrode for 3 min, unless otherwise stated. A 0.5 M pyrrole solution containing 4000 mg l^{-1} of urease was used (unless otherwise stated) for the electropolymerisation. A three-electrode electrochemical cell was used for the electropolymerisation. A platinum disc or cylindrical electrode (2 mm diameter and 1 cm length) was used as the working electrode, while a Pt electrode and an Ag/AgCl electrode were employed as the auxiliary and reference electrodes, respectively.

Characterisation of the enzyme in the polypyrrole film was achieved by cyclic voltammetry, chronopotentiometry, amino acid analysis and X-ray photoelectron spectroscopy, as previously described [10].

2.3. Instrumentation

A potentiostat/galvanostat designed and built within the Faculty of Science and Technology at the University of Western Sydney, Nepean, was employed for the electropolymerisation. The galvanostat was connected to a computer and an Epson LX-400 printer.

The amperometric measurements were performed using a working electrode (containing a polypyrrole–urease film), a reference electrode (Ag/AgCl), and a Pt auxiliary electrode. A BAS CV-27 connected to an ICI chart recorder (DP 600 Dual Pen Recorder) was used for the dc-amperometric detection, while a Dionex pulsed-amperometric detector (PAD-2) was used for the pulsed-amperometric detection. In the latter case, a repetitive, pulsed potential waveform is employed and the current is sampled at an appropriate point on the potential pulse. Flow injection analysis was performed on an ICI HPLC pump (LC 1500), as previously described [3].

2.4. Sampling and pretreatment procedures

Blood samples were collected and treated after separating the blood plasma from the red blood cells by the staff of a local hospital (Westmead Hospital). Prior to performing FIA, the blood plasma samples were diluted to 1 : 100 and the undesirable anions were removed on-line by passing through an anion-exchange column, as shown in Fig. 1. The blood plasma samples were analysed for urea (Nepean) employing FIA with amperometric detection (dc and pulsed) on the PPy–urease biosensor at our laboratory, and employing a standard spectrophotometric method at the hospital.

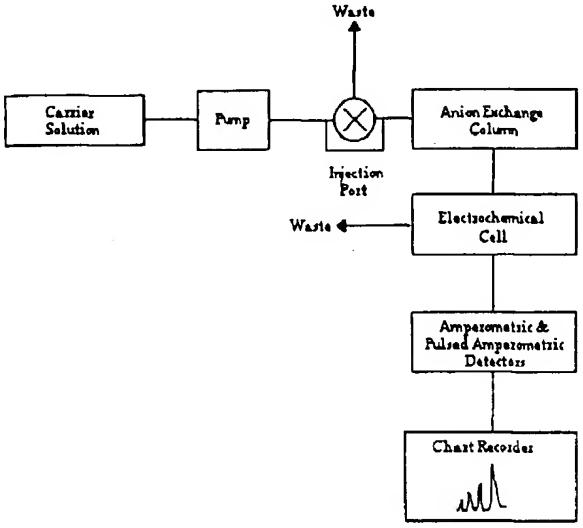

Fig. 1. Instrumentation used for in situ anion exchange separation and FIA of urea on PPy–urease biosensor with dc and pulsed amperometric detection.

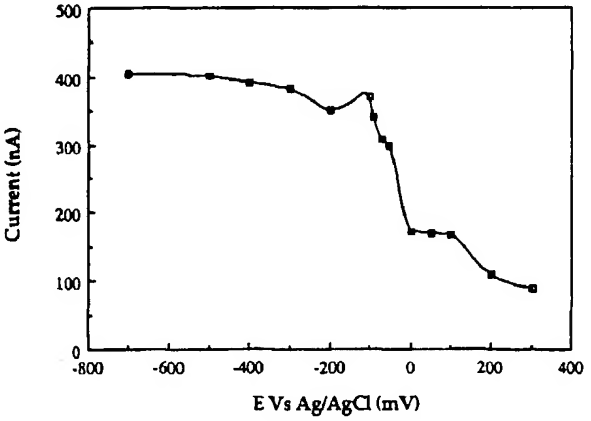

Fig. 2. Influence of final pulsed potential (E_2) on amperometric detection of urea on PPy–urease biosensor using FIA. Film formation conditions: 4 g l^{-1} urease, 0.5 M pyrrole and i_{app}=0.5 mA cm^{-2} for 1 min. Conditions for amperometric measurement: Pulse width 120 mS, E_1=−70 mV vs Ag/AgCl, 0.05 M phosphate buffer, pH 7.0, 0.01 M urea (in buffer) and flow rate=0.1 ml min^{-1}.

3. Results and discussion

3.1. Pulsed-amperometric detection

We have demonstrated in a recent study [3] that the determination of urea on a PPy–urease biosensor can be reliably performed employing FIA with dc-amperometric detection in 0.05 M phosphate buffer (pH 7.0), an applied potential of −70 mV vs Ag/AgCl and a flow rate of 0.1 ml min^{-1}. The influence of flow rate on the FIA of urea was also discussed in that paper. The achievable detection limit of the dc-amperometric detection mode was 3 mg l^{-1}. However, it has recently been demonstrated in other studies [12,13] that the use of pulse amperometric detection is useful in improving the detection limit for the FIA of various substances. The use of the pulsed-amperometric detection is considered in the present study for improving the detection limit of the PPy–urease biosensor for urea and its application to blood samples.

Fig. 2 shows that the application of pulses during the measurements had some effects on the amperometric response of urea, depending on the direction of the pulse. The application of a positive potential pulse from −70 mV (E_1) to more positive potentials (E_2) results in the reduction of the sensitivity of the amperometric response, whereas the use of negative pulses improved the sensitivity of the urea response. In this detection mode, the current is sampled at the end of the pulse potential (E_2). The variation in E_2 in the range −700 to +300 mV, as shown in Fig. 2, results in a considerable reduction in the flow amperometric response at more positive potentials than −100 mV. The optimum response for urea was obtained with the application of negative potential pulses in the range −70 (E_1) to −400 mV (E_2).

The influence of pulse width on the amperometric response of urea is shown in Fig. 3. The sensitivity of the urea response increased when the pulse width was increased from 60 to 120 mS, but remained fairly constant at longer pulse widths. The use of longer pulse widths, in the range 120–250 mS, gave the optimum response for urea. This may be due to a slow catalytic reaction at the electrode surface.

3.2. Analytical characteristics of PPy–urease biosensor with pulsed detection

The flow amperometric response obtained for the PPy–urease biosensor with pulsed detection of urea was very stable, even with continuous use for two weeks. Fig. 4 shows the typical flow amperograms

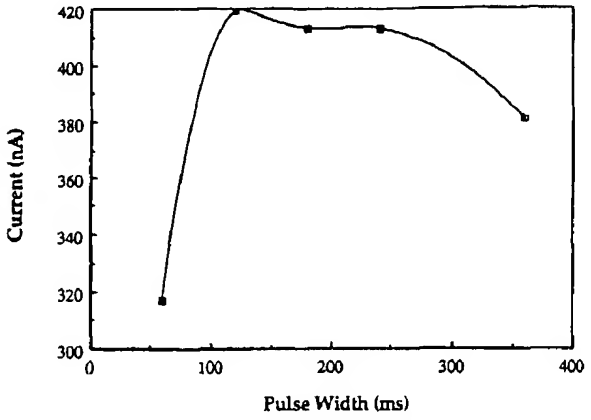

Fig. 3. Influence of pulse width on amperometric response of urea. Experimental conditions were the same as for Fig. 2, except: E_1=−70 mV and E_2=−400 mV vs Ag/AgCl, pulse width was varied.

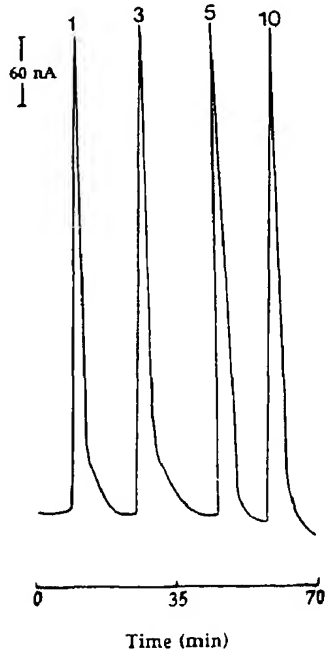

Fig. 4. Stability of pulsed amperometric response of urea obtained using FIA on the PPy–urease biosensor after two weeks of continuous use. All experimental conditions were same as for Figs. 2 and 3, except that 0.1 mM urea was used. Number indicate ten repeated injections.

obtained for ten repeated injections of urea on the biosensor after two weeks of continuous use. Evidently, the pulsed-amperometric measurement was highly reproducible with a 1% rsd. It appears that this mode of detection causes minimum disruption to the PPy–urease film and, thus, prolongs the stability of the biosensor.

A linear concentration range, 100–4500 μg l^{-1} (correlation coefficient=0.987), was obtained for urea with a detection limit of 60 μg l^{-1}. However, a more linear range was evident in the range 100–3000 μg l^{-1} (correlation coefficient=0.998). To our knowledge, the detection limit obtained in this study is the lowest reported for the urease biosensor to date. This value is considerably lower than the minimum detectable amount, 1 mg l^{-1}, reported for naturally immobilised urease [14] or, 3 mg l^{-1}, reported for urease coupled to ammonia electrode [15]. Also, the response of urea obtained with the pulsed-amperometric detection on PPy–urease is five times more sensitive than those obtained with dc-amperometric detection [3]. In comparison, the detection limit achieved by flow injection analysis on PPy–urease with the dc-amperometric detection was 3 mg l^{-1} and the linear concentration range was 3–15 mg l^{-1}. Furthermore, the use of the dc-amperometric detection mode required fresh (daily) film formation. Evidently, the use of the pulsed-amperometric detection is beneficial for the reliable determination of very low concentrations (0.1–4.5 mg l^{-1}) of urea, while dc-amperometric detection is suitable for higher concentrations (>5 mg l^{-1}).

The application of the FIA method with pulsed-amperometric detection in the determination of urea in simulated samples proved to be successful. The concentrations of urea considered in the recovery study were comparable to those found in the diluted blood samples, as described in Section 2. The data in Table 1 indicate that a recovery in the range 95%–112% is obtained at different analyte concentrations. As can be observed from this table, the reproducibility

Table 1
Recovery of urea in simulated samples with pulsed amperometric detection on the polypyrrole–urease biosensor

Amount added (mg l^{-1})	Amount found by biosensor [a] (mg l^{-1})	Percentage of recovery [b]
0.6	0.57±0.09	95±17%
3.0	3.3±0.3	109±9%
6.0	6.7±0.2	112±3%

n=10, error is [a] standard deviation or [b] relative standard deviation.

of the recovery efficiency improved with increasing concentration of urea. A relatively high reproducibility of 3% rsd was obtained with the biosensor for the recovery of ≥ 6 mg l^{-1} of urea.

3.3. Application to blood plasma

The amperometric response obtained for urea on the PPy–urease biosensor by direct injection of the blood plasma was severely affected by matrix interferences. The dilution (1 : 100) of the blood plasma reduced the amount of interference significantly, but did not completely eliminate the positive interference. The anion-exchange removal of possible interferants, such as anions and proteins, from the blood plasma prior to the amperometric detection, as illustrated in Fig. 1, proved to be suitable for the reliable determination of urea on the biosensor. Nevertheless, it was necessary to increase the thickness of the PPy–urease film and increase the flow rate to 0.3 ml min^{-1} in order to improve the stability of the biosensor. The reduced interaction of the blood plasma with the polymer film under these conditions might have been responsible for the improved performance of the biosensor. We have shown in a previous study [10,11] that an increase in the film thickness, accomplished by increasing the polymerisation period from 1 min to 3 min, does not alter the analytical characteristics of the biosensor significantly. However, this has proven to be useful in minimising the interference from the blood plasma components in this study.

Fig. 5 shows typical responses obtained from the FIA of urea in blood plasma on the biosensor with the anion-exchange separation and dc-amperometric detection. Quantification of urea in the samples (peaks d–i) was based on the use of a calibration curve constructed from the peaks a–c. The pulsed-amperometric detection of urea in blood plasma by FIA with the anion-exchange separation was also successful.

Table 2 provides the results obtained for urea in blood samples on the PPy–urease biosensor by FIA with dc- and pulsed-amperometric detection. The data show that the results obtained on the biosensor for urea in the blood plasma samples were in good agreement with those for the standard spectrophotometric method.

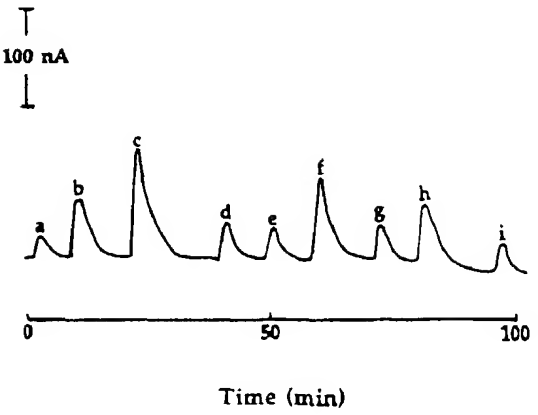

Fig. 5. Flow amperograms obtained for FIA of urea in blood plasma on PPy–urease biosensor with in situ anion exchange separation and dc amperometric detection. All conditions were the same as for Figs. 2 and 3, except polymer was grown for 3 min and flow rate was 0.3 ml min^{-1}. Urea standard responses: (a) 0.01, (b) 0.1 and (c) 1mmol; sample responses: (d–i).

Table 2
Urea concentrations found in blood plasma by a spectrophotometric method and with the polypyrrole-based amperometric biosensors

Blood plasma sample	[Urea][a] mM	[Urea][b] mM
		dc
C 16208	1.4	1.7
C 16206	3.6	3.5
C 16207	2.3	2.5
C 16211	2.1	2.1
C 16212	14.7	14.5
C 16205	6.8	6.5
		pulsed
C 24589	4.6	5.3
C 24920	5.4	5.6
C 24917	8.7	7.8
C 24892	3.9	3.6
C 24675	30.2	25.9
C 24843	4.4	5.3

[a] Spectrophotometric method; [b] FIA with PPy–urease biosensor.

4. Conclusion

The use of pulsed-amperometric detection with PPy–urease biosensor for the FIA determination of urea has been successfully demonstrated. Substantial improvements in the sensitivity, detection limit and linear concentration range were accomplished using the pulsed-amperometric detection mode. A much

wider linear concentration range (0.1–4.5 mg l^{-1}) and a relatively low detection limit (60 μg l^{-1}) was obtained by this method. Also, excellent recovery (95%–112%) of urea was accomplished for the simulated samples. The removal of the interferants by an anion-exchange separation was necessary for the reliable determination of urea in blood plasma. The results obtained with the biosensor agreed favourably with a standard spectrophotometric method.

Acknowledgements

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L2 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2001:720551 CAPLUS
DN 136:306161
ED Entered STN: 03 Oct 2001
TI Oxygen effects on glucose meter measurements with **glucose dehydrogenase**- and oxidase-based test strips for point-of-care testing
AU Tang, Zuping; Louie, Richard F.; Lee, Judith H.; Lee, Debra M.; Miller, Earl E.; Kost, Gerald J.
CS Point-of-Care Testing Center for Teaching and Research, University of California, Davis, CA, USA
SO Critical Care Medicine (2001), 29(5), 1062-1070
CODEN: CCMDC7; ISSN: 0090-3493
PB Lippincott Williams & Wilkins
DT Journal
LA English
CC 9-1 (Biochemical Methods)
Section cross-reference(s): 14
AB Objectives: To determine the effects of different oxygen tensions (Po2) on glucose measurements with **glucose dehydrogenase** (GD)-based and glucose oxidase (GO)-based test strips, to quantitate changes in glucose measurements observed with different Po2 levels, and to discuss the potential risks of oxygen-derived glucose errors in critical care. Design: Venous blood from healthy volunteers was tonometered to create different oxygen tensions simulating patient arterial Po2 levels. Venous blood from diabetic patients was exposed to air to alter oxygen tensions simulating changes in Po2 during sample handling. Whole-blood glucose measurements obtained from these samples with six glucose meters were compared with reference analyzer plasma glucose measurements. Glucose differences were plotted vs. different Po2 levels to identify error trends. Error tolerances were as follows: (a) within ±15 mg/dL of the reference measurement for glucose levels ≤100 mg/dL; and (b) within ±15% of the reference measurement for glucose levels >100 mg/dL. Setting and Subjects: Five healthy volunteers in the bench study and 11 diabetic patients in the clin. study. Results: In the bench study, increases in Po2 levels decreased glucose measured with GO-based amperometric test strips, mainly at Po2 levels >100 torr. At nearly constant glucose concns., glucose meter systems showed large variations at low (39 torr) vs. high (396 torr) Po2 levels. Glucose measured with GD-based amperometric and GO-based photometric test strips generally were within error tolerances. In the clin. study, 31.6% (Precision PCx), 20.2% (Precision QID), and 23.0% (Glucometer Elite) of glucose measurements with GO-based amperometric test strips, 14.3% (SureStep) of glucose measurements with GO-based photometric test strips, and 4.6% (Accu-Chek Advantage H) and 5.9% (Accu-Chek Comfort Curve) of glucose measurements with GD-based amperometric test strips were out of the error tolerances. Conclusions: Different oxygen tensions do not significantly affect glucose measured with the GD-based amperometric test strips, and have minimal effect on GO-based photometric test strips. Increases in oxygen tension lowered glucose measured with GO-based amperometric test strips. The authors recommend that the effects of different oxygen tensions in blood samples on glucose measurements be minimized by using **oxygen-independent** test strips for point-of-care glucose testing in critically ill and other patients with high or unpredictable blood Po2 levels.
ST oxygen **glucose** meter **dehydrogenase** oxidase test strip